

Amendments to the Claims:

This listing of the claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1 (Currently Amended). A method of altering gene expression in a population of human embryonic stem cells, with a transfection efficiency greater than that obtainable by means of electroporation using a single 625 V/cm pulse at room temperature, without affecting the pluripotent character of the cells, comprising:

introducing a polynucleotide into the population of human embryonic stem cells by transfection in the presence of at least one cationic non-lipid polymer transfection reagent, ~~selected from the group consisting of a cationic non-lipid polymer reagent, a non-liposomal reagent, and a cationic lipid agent,~~ wherein said polynucleotide contains a gene expression altering sequence so that gene expression in the embryonic stem cells after introducing the polynucleotide becomes measurably different from gene expression prior to introducing the polynucleotide, wherein the cationic non-lipid polymer transfection reagent is one that provides a transfection efficiency greater than that obtainable by electroporation using a single 625 V/cm pulse at room temperature, and wherein the

polynucleotide introduced into the human embryonic stem cells does not contain viral genes.

2 (Previously Presented). The method according to claim 1, wherein the expression altering sequence is an enhancer sequence for modulating gene expression in the population of embryonic stem cells.

3 (Previously Presented). The method according to claim 1, wherein the expression altering sequence is a gene encoding a protein and said protein is not expressed in the population of embryonic stem cells in the absence of the polynucleotide.

4 (Previously Presented). The method according to claim 3, wherein the protein is selected from the group consisting of a fluorescent protein and an antibiotic resistance protein.

5 (Previously Presented). The method according to claim 4, wherein the fluorescent protein is selected from the group consisting of green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan protein and yellow cyan protein.

6 (Previously Presented). The method according to claim 4, wherein the antibiotic resistance protein is selected from the group consisting of hygromycin, neomycin, zeocin and puromycin.

7 (Currently Amended). The method according to claim 1, wherein said ~~transfection reagent is a~~ cationic non-lipid polymer transfection reagent is a linear polymer of ethyleneimine.

8-10 (Cancelled)

11 (Currently Amended). A method of altering gene expression in a population of human embryonic stem cells, with a transfection efficiency greater than that obtainable by means of electroporation using a single 625 V/cm pulse at room temperature, comprising:

introducing a DNA sequence into the population of human embryonic stem cells by transfection in the presence of a cationic non-lipid polymer transfection reagent, wherein said DNA sequence corresponds to at least one of an enhancer, a promoter, and a gene so as to alter gene expression in the population of embryonic cells in an amount to permit cells containing the DNA sequence to be distinguished from cells absent the DNA sequence, wherein the cationic non-lipid polymer transfection reagent is one that provides a transfection efficiency greater than that obtainable by electroporation using a single 625 V/cm pulse at room temperature, and wherein the DNA sequence introduced into the human embryonic stem cells does not contain viral genes.

12 (Previously Presented). The method according to claim 11, wherein the DNA sequence corresponds to a gene and the gene encodes a protein selected from the group consisting of a fluorescent protein, a suicide gene, and an antibiotic resistance protein.

13 (Previously Presented). The method according to claim 11, wherein the DNA sequence comprises a promoter selected from the group consisting of rex-1, oct-4, oct-6, SSEA-3, SSEA-4, TRA1-60, TR1-81, GCTM-2, alkaline phosphatase, and Hes 1 promoters.

14 (Previously Presented). The method according to claim 12, wherein the fluorescent protein is selected from the group consisting of green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan protein and yellow cyan protein.

15 (Previously Presented). The method according to claim 12, wherein the protein is an antibiotic resistance protein and the antibiotic resistance protein is selected from the group consisting of hygromycin, neomycin, zeocin and puromycin.

16 (Previously Presented). The method according to claim 12, wherein the DNA corresponds to a suicide gene and the suicide gene is an inducible apoptotic gene or encodes a protein

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selected from the group consisting of inducible Diphtheria toxin, and bacterial cytosine deaminase.

17 (Previously Presented). The method according to claim 11, wherein the DNA sequence causes a knockout of a genomic sequence, the genomic sequence being selected from the group consisting of beta 2 microglobulin, HLA-1, HLA-2 and an INF receptor gene sequence.

18-56 (Cancelled)

57-58 (Not Entered)

59 (Previously Presented). The method according to claim 1, further comprising selecting and verifying that the population is a substantially pure population of stably transfected pluripotent hES cell with the gene expression altering sequence.

60 (Previously Presented). The method according to claim 7, wherein the expression altering sequence is a gene encoding a protein and said protein is not expressed in the population of embryonic stem cells in the absence of the polynucleotide.

61 (Previously Presented). The method according to claim 11, wherein said DNA sequence is a gene encoding a protein and said protein is not expressed in the population of embryonic stem cells in the absence of the DNA sequence.

62-64 (Cancelled)

65 (Currently Amended). A method for transfecting human embryonic stem cells without affecting the pluripotent character of the cells, comprising:

transfecting human embryonic stem cells with a polynucleotide in the presence of at least one cationic non-lipid polymer transfection reagent, ~~selected from the group consisting of a cationic non-lipid polymer reagent, a non-liposomal reagent, and a cationic lipid agent~~, wherein the transfection reagent is one that provides a transfection efficiency greater than that obtainable by electroporation using a single 625 V/cm pulse at room temperature, and wherein the polynucleotide introduced into the human embryonic stem cells does not contain viral genes.

66 (Currently Amended). The method according to claim 65, wherein said ~~transfection reagent is a~~ cationic non-lipid polymer transfection reagent is a linear polymer of ethyleneimine.

67 (Currently Amended). ~~A~~ The method ~~in accordance with~~ according to claim 65, wherein said polynucleotide comprises a gene encoding a protein and said protein is not expressed in the population of embryonic stem cells in the absence of the polynucleotide.

68 (Previously Presented). The method according to claim 67, wherein the protein is selected from the group

consisting of a fluorescent protein and an antibiotic resistance protein.

69-70 (Cancelled)

71 (New). The method according to claim 11, wherein said cationic non-lipid polymer transfection reagent is a linear polymer of ethyleneimine.

72 (New). A method of altering gene expression in a population of human embryonic stem cells, without affecting the pluripotent character of the cells, comprising:

introducing a polynucleotide into the population of human embryonic stem cells by transfection in the presence of transfection reagent that is a linear polymer of polyethyleneimine, wherein said polynucleotide contains a gene expression altering sequence so that gene expression in the embryonic stem cells after introducing the polynucleotide becomes measurably different from gene expression prior to introducing the polynucleotide, and wherein the polynucleotide introduced into the human embryonic stem cells does not contain viral genes.

73 (New). A method of altering gene expression in a population of human embryonic stem cells, comprising:

introducing a DNA sequence into the population of human embryonic stem cells by transfection in the presence of a transfection reagent that is a linear polymer of

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polyethyleneimine, wherein said DNA sequence corresponds to at least one of an enhancer, a promoter, and a gene so as to alter gene expression in the population of embryonic cells in an amount to permit cells containing the DNA sequence to be distinguished from cells absent the DNA sequence, and wherein the DNA sequence introduced into the human embryonic stem cells does not contain viral genes.

74 (New). A method for transfecting human embryonic stem cells without affecting the pluripotent character of the cells, comprising:

transfecting human embryonic stem cells with a polynucleotide in the presence of at least one transfection reagent that is a linear polymer of polyethyleneimine, wherein the polynucleotide introduced into the human embryonic stem cells does not contain viral genes.